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Bitter taste receptors: Novel insights into the biochemistry and pharmacology

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ABSTRACT

Bitter taste receptors (T2Rs) belong to the super family of G protein-coupled receptors (GPCRs). There are 25 T2Rs expressed in humans, and these interact with a large and diverse group of bitter ligands. T2Rs are expressed in many extra-oral tissues and can perform diverse physiological roles. Structure-function studies led to the identification of similarities and dissimilarities between T2Rs and Class A GPCRs including amino acid conservation and novel motifs. However, the efficacy of most of the T2R ligands is not yet elucidated and the biochemical pharmacology of T2Rs is poorly understood. Recent studies on T2Rs characterized novel ligands including blockers for these receptors that include inverse agonist and antagonists. In this review we discuss the techniques used for elucidating bitter blockers, concept of ligand bias, generic amino acid numbering, the role of cholesterol, and conserved water molecules in the biochemistry and pharmacology of T2Rs.

1. Introduction

1.1. G protein-coupled receptors

G protein-coupled receptors (GPCRs) are the largest known family of membrane receptors in eukaryotes comprised of more than 800 members. These receptors recognize a wide range of extracellular stimuli such as photons, hormones, neurotransmitters, tastants and odorants to induce intracellular signaling pathways (Billington and Penn, 2003; Schoneberg et al., 2004; Takeda et al., 2002). More than 50% of the drugs available in the market directly or indirectly target GPCRs, highlighting their significance in a variety of biological mechanisms (Tautermann, 2014).

1.2. Taste perception

Taste perception is an important chemosensory function of the gustatory system that evaluates the quality of food, and avoids ingestion of poisonous substances (Herness and Gilbertson, 1999; Wu et al., 2002). Humans can recognize five basic tastes namely sweet, umami, bitter, salt and sour. Among these tastes, bitter perception is believed to play a critical role in defense mechanisms against numerous toxic substances acting as a central warning signal (Wu et al., 2002). Bitter perception is mediated by bitter taste receptors (T2Rs) that are expressed in the oral cavity. There are 25 T2Rs expressed in humans that belong to the GPCR superfamily (Chandrashekar et al., 2000). However, T2Rs share low sequence similarity with other GPCRs and their classification is still ambiguous although they are considered as a separate group of receptors (Di Pizio and Niv, 2015; Sharman et al., 2013). In this article we review recent developments in the structure-function analysis and the pharmacology of bitter taste receptors (T2Rs). A review on the extra-oral functions of T2Rs and their pathophysiological roles are discussed in the accompanying article in the current issue (Shaik et al., 2016).

Abbreviations: GPCRs, G protein-coupled receptors; T2Rs, bitter taste receptors; CAMs, constitutive active mutants; TM, transmembrane; PLCβ_2, phospholipase Cβ_2; IP_3, inositol-1,4,5-triphosphate; ECL, extracellular loop; ICL, intracellular loop.

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1.3. Signal transduction by T2Rs in gustatory and non-gustatory tissues

Bitter taste signaling is initiated by a bitter agonist's interaction with its cognate T2R. This is hypothesized to induce a conformational change in the receptor and subsequent activation of intracellular heterotrimeric G-protein complex (Chaudhari and Roper, 2010). In gustatory tissues, it was reported that the heterotrimeric complex comprises of $G\alpha_{gustducin}$ and $\beta_3\gamma_{13}$ subunits (Caicedo et al., 2003; Wong et al., 1996). Once activated, $G\alpha_{gustducin}$ separates from the complex and activates a phosphodiesterase to reduce cAMP levels while the $\beta_3\gamma_{13}$ dimer activates phospholipase $C\beta_2$ ($PLC\beta_2$) (Caicedo et al., 2003). $PLC\beta_2$ acts on membrane bound phosphatidylinositol-4,5-diphosphate (PIP_2) to generate inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). The generated IP_3 activates IP_3 specific receptors (IP_3R) present on endoplasmic reticulum to release calcium (Ca^{2+}) (Chaudhari and Roper, 2010). The elevated Ca^{2+} opens transient receptor potential cation channel member 5 (TRPM5) that leads to membrane depolarization and subsequent neurotransmitter release to send signal to the brain (Hofmann et al., 2003). However, in non-gustatory tissues such as the airway smooth muscle cells, it was proposed that T2Rs activation stimulates two opposing Ca^{2+} signaling pathways mediated by $\beta\gamma$ subunits (Zhang et al., 2013). The *in vivo* studies on $\alpha_{gustducin}$ knockout mice demonstrated a notable sensitivity to bitter compounds indicating unknown mechanisms in bitter taste signaling other than $\alpha_{gustducin}$ pathway (Caicedo et al, 2003; Wong et al., 1996).

2. Pharmacology of bitter ligands

2.1. Bitter agonists

The extra-oral expression of T2Rs strongly suggests for the existence of endogenous ligands, which remains to be elucidated. However, a wide range of structurally diverse compounds can activate T2Rs. More than 700 compounds were suggested to taste bitter while the efficacy of most of these compounds for the different T2Rs remains to be determined, pharmacologically (Devillier et al., 2015; Wiener et al., 2012). These bitter agonists include plant-derived and synthetic compounds such as amides, peptides, heterocyclic compounds, glycosides, alkaloids, terpenoids, phenols and flavonoids (Meyerhof et al., 2010; Pronin et al., 2004). Many pharmaceutical compounds like quinine, chloroquine, erythromycin and ofloxacin, and bacterial stimulants like acyl homoserine lactones are also known to activate T2Rs (Tizzano et al., 2010). Many T2Rs can be activated by multiple bitter molecules, while few T2Rs can only recognize a single compound (Behrens et al., 2009; Brockhoff et al., 2007; Sakurai et al., 2010b). This was extensively studied using activation profile for each T2R in HEK293T cells (Meyerhof et al., 2010). Although the bitter agonists are structurally diverse, some T2Rs exhibit selectivity towards the compounds with conserved moieties and position of reactive groups. For example, compounds with β-D-glucopyranoside moiety activate T2R16 and isothiocynates activate T2R38 (Sanematsu et al., 2014). A more recent study analyzed the promiscuity and selectivity of both bitter ligands and human T2Rs (Di Pizio and Niv, 2015). In this report they proposed that promiscuous bitter compounds activate all the selective T2Rs while both selective and promiscuous compounds can activate promiscuous T2Rs. However, no compound is known to activate all 25 T2Rs or no unique compound towards a selective T2R (Di Pizio and Niv, 2015; Meyerhof et al., 2010).

In this report, we review the known bitter agonists to date as well as their activation or threshold concentrations (TC) with respect to the T2Rs (Supplementary Table 1). Among the 21 deorphanized T2Rs, T2R10, T2R14 and T2R46 are broadly tuned and exhibit a wide receptive range in recognizing structurally different molecules (Brockhoff et al., 2011). Additional ligands were recently discovered for T2R14 (Levit et al., 2014). TAS2R gene expression studies in different tissues showed that these receptors are expressed from moderate to high levels. Thus, consideration of the relatively high expression of these receptors in conjunction with their wide receptive range may suggest an important role of these three receptors in non-gustatory tissues.

Until recently, T2R41 was considered as one of the orphan receptors. Recent investigations showed that chloramphenicol can activate T2R41-P127 and L127 variants with a TC of 300 μM and 600 μM respectively (Thalmann et al., 2013). Another study identified 4-Fluorophenyl biguanide as T2R41 agonist with EC_{50} value of 1.37 ± 0.02 mM (Ji et al., 2014). A more extensive study on 97 compounds comprising flavonoids and isoflavonoids have identified 68 compounds activating T2R14, and 70 compounds activating T2R39 with an overlap of 58 compounds activating both the T2Rs between the threshold values ranging from 0.12 to 500 μM (Roland et al., 2013). This study also determined EC_{50} values for some of the identified compounds. However, until now, endogenous agonists for T2Rs are not known and all the known agonists are exogenous compounds prepared synthetically or derived from plants and bacteria.

2.2. Bitter antagonists and inverse agonists (bitter taste blockers)

In simple pharmacological terms an antagonist is considered as a ligand that does not induce any biological response from a GPCR, while a ligand that reduces the basal level activity of a GPCR is called an inverse agonist. These compounds act as competitive or allosteric inhibitors to block the receptor activity. For T2Rs these compounds are referred to as bitter taste blockers or bitter blockers. Until now, only 13 bitter blockers have been identified (Brockhoff et al., 2011; Fletcher et al., 2011; Greene et al., 2011; Pydi et al., 2015, 2014c; Roland et al., 2014; Slack et al., 2010). However, likewise with agonists, none of these blockers can block all the 25 T2Rs. They interact with only 10 T2R subtypes (Table 1). A recent analysis suggested that agonist-to-antagonist ratio in T2Rs is larger than in Class A GPCRs and thus a need for the characterization of more antagonists (Di Pizio et al., 2016).

The use of bitter taste maskers is a common practice in food and beverage industries to reduce the bitterness and increase palatability (Ley, 2008). Some of the bitter maskers used in food and pharmaceutical industries are summarized in Table 2. Although these compounds are known to mask the bitterness, the T2Rs they interact with and their biochemical pharmacology have not been elucidated. With the recent characterization of extra-oral expression of T2Rs, it is essential to study the interaction of these maskers with T2Rs and identify novel bitter blockers. These bitter blockers have been proposed to have at least three main advantages. First, bitter blockers will increase the palatability of bitter tasting food and beverages; second, they increase the compliance of bitter tasting drugs, especially children's formulations; and finally reduce or prevent off-target drug effects in the extra-oral tissues (Clark et al., 2012). Thus, the discovery of more potent and selective antagonists is required to overcome these challenges.

GIV3727 (4-(2,2,3-trimethylcyclopentyl) butanoic acid) was the first T2R antagonist discovered by high-throughput screening of 17,854 compounds (Slack et al., 2010). It acts as an orthosteric insurmountable antagonist for T2R31 with an IC_{50} value of 6.4 ± 2.4 μM against acesulfame K. This study was followed by the discovery of sesquiterpene lactones and probenecid as bitter taste blockers (Brockhoff et al., 2011; Greene et al., 2011). Probenecid is originally known as an inhibitor of organic anion transporter channels which is mainly used as a uricosuric drug